

Tar intein incubation protocol

Goal: To check the intein splicing

Protocol:

5 ml O/N starter in TB to:

*Tar1 (+Amp)

*UU

*Tar1 intein1 (+Amp)

*Tar1 intein2 (+Amp)

1. Dilute 1:50 and growth in 30°C to O.D(600nm) ~0.6* (Erlenmeyer 250 ml)
. **Total volume:** 50 µl.
2. Divide to 5 tubes, **5 ml** each (falcon 50 ml). (total of 20 tubs)
3. Dilution with the bacteria: 0, 10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵.

Cons. 10 ⁻⁵	Cons. 10 ⁻⁴	Cons. 10 ⁻³	Cons. 10 ⁻²	Cons. 10 ⁻¹	
4.5 ml	4.5 ml	4.5 ml	4.5 ml	5.5 ml	Bactria
500µl	500µl	500 µl	500 µl	55 µl* β-estradiol fresh (C=10 mg/ml)	Bactria with β-estradiol

1. Move to 32C for 2 hr (for intein splicing).
- 2.
3. **-NOTE:** Meanwhile make plats according to "swarming assay" protocol.
4. After 2 hr do "swarming assay".

*according to the article (the assay: "Chemical detection with strain DIER and the YES system").